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Physicochemical Properties of Indonesian Pigmented Rice (*Oryza sativa* Linn.) Varieties from South Sulawesi

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ABSTRACT

Pigmented Rice (PR) is one of the valuable food crops used in indigenous medicine. Thirteen PR varieties were collected from 3 climatic zones of South Sulawesi, Indonesia and analyzed for grain dimension, color and proximate composition. The PR were extracted using acidic ethanol and then measured their total phenolics, anthocyanins and 2,2-diphenyl-1-picrylhydrazyl (DPPH) scavenging activity. Grain size determination showed that the length of the rice was in the range of 5.60-6.82. There was no significant different ($p>0.05$) in rice length and length/width ratio. The colorimetric results indicated that the darkest black glutinous rice had the lowest brightness parameter L^* value which was negatively correlated with its total anthocyanin ($r = -0.673$) and phenolic ($r = -0.559$) contents. Proximate analysis results showed that all of PR varieties fulfilled the water content limit ($\leq 14\%$). The total contents of ash, fat, protein, crude fiber and carbohydrate were in the range of 1.19-2.13, 1.06-3.05, 7.24-14.02, 0.66-0.99 and 71.29-77.14%, respectively. The anthocyanin and phenolic contents of black glutinous rice extracts were in the range of 94.70-202.46 mg Cy-3-glc/100 g db and 292.74-746.25 mg GAE/100 g db, respectively, which were higher than the black rice (66.08-113.83 mg Cy-3-glc/100 g db and 119.74-230.10 mg GAE/100 g db) and the red rice (0-12.85 mg Cy-3-glc/100 g db and 12.52-64.52 mg GAE/100 g db). Antioxidant activity was positively correlated with total phenolic ($r = 0.886$) and anthocyanin ($r = 0.650$) compounds. The data suggests the potency of black glutinous rice as a part of nutraceutical product formulation.

Key words: Pigmented rice, South Sulawesi, antioxidant, anthocyanin, phenols

INTRODUCTION

Rice is a strategic commodity in Indonesia, not only as a primary food crop cultivated by most of the Indonesian farmer but also as a staple food for more than 240 million of

Indonesian peoples (Statistics Indonesia, 2012; Panuju *et al.*, 2013). Indonesia is the third biggest rice producer in the world after China and India and South Sulawesi is the largest crop-producing regions in the eastern of Indonesia (FAOSTAT., 2012; Statistics Indonesia, 2012).

Pigmented rice is one of the materials widely used by Buginese and Makassarese people, 2 main tribes in South Sulawesi as food and traditional medicine, such as anti-diabetes mellitus, anti-asthma, post-partum treatment, anti-gastritis and skin care formulas. Pigmented rice has been reported to contain pigments, gammaoryzanol, phytic acid, vitamins, minerals, amino acids and the other compounds. The majority of the compounds are antioxidant components (Chakuton *et al.*, 2012; Abdel-Aal *et al.*, 2006; Park *et al.*, 2008) and responsible to the rice properties as an antimutagenic (Sadapod *et al.*, 2010), anti-lipid peroxidation (Sangkitikomol *et al.*, 2010a); ROS and NO suppressor (Hu *et al.*, 2003), anti-inflammatory (Saenjum *et al.*, 2012), aldose reductase inhibitor (Yawadio *et al.*, 2007), cholesterol absorption inhibitor (Yao *et al.*, 2013) and anti-hepatic steatosis (Jang *et al.*, 2012).

There are many traditional cultivars of pigmented rice in South Sulawesi. The pigmented rice is varied in flavor, color, physicochemical character and nutrition composition depending on monsoon climate of rice crop area (Herniwati and Kadir, 2009). Yet, the experiments about pigmented rice is not as much as white rice in Indonesia (Shinta *et al.*, 2014). Therefore, the aim of this study was to analyze the physicochemical properties of selected pigmented rice originating from western, eastern and transition zone of South Sulawesi climatic zones. Color, proximate content, total polyphenol, anthocyanin and antioxidant activity were parameters measured in this study.

The results of this study can be useful in promoting pigmented rice varieties from South Sulawesi as a functional food.

MATERIALS AND METHODS

Preparation of samples: Thirteen of pigmented rice were collected from 2 districts in western zone, 2 districts in eastern zone and 1 district in transition zone of South Sulawesi. Rice seed was collected in the unhusked form and pounded to remove the husk before being analyzed.

Grain Dimensions and Shape: Thirteen of rice seed samples were randomly collected. The dimensions were measured using dial micrometer to obtain the average length and width of rice seeds.

$$\text{Length to width ratio (L/W)} = \frac{\text{Rice average length (mm)}}{\text{Rice average width (mm)}}$$

Based on the L value, the length of the husked rice was categorized by IRRI as follows: extra long = >7.50 mm, long = 6.61-7.50 mm, medium = 5.51-6.60 mm and short = 4.50 mm. Meanwhile, grain shape was characterized based on the L/W ratio: slender >3.0, medium 2.1-3.0, bold 1.1-2.0 and round 1.0 or less (Juliano, 1993; Cruz and Khush, 2000).

Color analysis: About 100 g pigmented riced was placed on a flat container with size 10×10 cm². Samples were set until the bottom of container covered. The color intensity was measured by attaching colorimeter TES 135 to the samples. It produced three color parameters i.e brightness (L*), redness (a*) and yellowness (b*). Prior to the assessment, calibration of the colorimeter was performed by using standardized white color in the instrument. Replication of examination were ten times for each samples (Yodmanee *et al.*, 2011).

Extraction: Pigmented rice was cleaned and crushed to a coarse powder. About 20 g pigmented rice were extracted with 50 mL HCl-ethanolic solution (HCl 0.01% in ethanol 96%). Maceration was done with modification using sonicator for 30 min, 40°C. The extract was collected and filtered. Filtration and re-extraction was conducted until a colorless solution obtained with total volume of the pigment about 800 mL. A part of rice pigment was used to analyze its anthocyanin content and the other was rotary evaporated at 50°C. The extract was subsequently placed in cool temperature in refrigerator.

Yield: The extraction yield was calculated based on the dry weight of the sample used (the unit of g/100 g dry basis of the rice).

$$\text{Yield (\%, w/w)} = \frac{\text{Weight of rice extract after evaporation}}{\text{Weight of rice powder before extraction}} \times 100$$

Determination of total phenolic contents: Total phenolic compounds of the HCl-ethanolic extracts were determined by the Folin-Ciocalteu method (Kumar *et al.*, 2008; Males *et al.*, 2006). A standard curve was constructed using various concentrations of gallic acid (Merck). The results were expressed as percentage of gallic acid.

Determination of anthocyanin contents: Anthocyanin of the pigmented rice extracts were conducted by pH differential method (Wrolstad, 1993; Giusti and Worldstat, 2001; Rodriguez-Saona and Wroistad, 2001). Anthocyanin concentration was calculated as cyanidin-3-glycosides using a molar extinction coefficient of 26900 L cm⁻¹ mg⁻¹ and a molecular weight of 445.2. The absorbance value is calculated using the equation:

$$A = [(A_{516} - A_{700}) \text{ pH } 1.0 - (A_{516} - A_{700}) \text{ pH } 4.5]$$

$$\text{Anthocyanin concentration (mg L}^{-1}\text{)} = \frac{A \times \text{MW} \times \text{DF} \times 1000}{\epsilon \times L}$$

Where:

A = Absorbance

MW = Molecular weight, molecular weight (445.2 g mol⁻¹)

DF = Dilution factor

ε = Molar extinction coefficient (26900 L cm⁻¹ mg⁻¹)

The anthocyanin concentration expressed in mg CYE/100 g dry bases of samples.
(CYE = cyanidin equivalent).

Determination of DPPH scavenging capacity: Radical scavenging activity was performed by using DPPH method (Zhang and Lin, 2008). The percentage of DPPH binding capacity of each pigmented extracts was calculated based on the formula:

$$\text{Scavenging of free radicals (\%)} = \frac{\text{Absorbance of control} - \text{absorbance of sample}}{\text{Absorbance of control}} \times 100$$

Each experiment was performed in triplicate, except proximate composition analysis.

RESULTS

Grain dimension and shape: The grain shapes of the collected PR varieties are given below in Table 1.

Thirteen of PR varieties had no significant differences in the length and L/W ratio ($p < 0.05$). The PR length was in the range of 5.60-6.82 mm. There were two PR varieties longer than the others. The L/W ratio was in the range of 2.22-2.90. It means that all rice had medium shape (between slender and bold).

Proximate analysis: In the development of a functional food product, it is important to understand the nature of each element in foodstuff. Although, the composition of foodstuffs is complex, the prominent elements in foodstuffs are water, minerals, carbohydrates, fats and proteins. The proximate analysis results of PR varieties are displayed in Table 2.

Color analysis: The color of the rice was determined using colorimeter. Pigment parameters (Table 3) consisted of $L^* = 19.75$ -50.03, $a^* = 2.30$ -15.75 and $b^* = -2.86$ -13.79 in range.

Table 1: Pigmented rice varieties from South Sulawesi

Rice varieties	L (cm)	Grain length	L/W ratio	Grain shapes
Black waxy rice				
A	0.681±0.0190	Long	2.10±0.13	Medium
C	0.6915±0.041	Long	2.82±0.18	Medium
E	0.614±0.0350	Medium	2.34±0.17	Medium
F	0.629±0.0410	Medium	2.39±0.23	Medium
I	0.661±0.0620	Long	2.77±0.32	Medium
J	0.610±0.0300	Medium	2.42±0.70	Medium
Red rice				
B	0.581±0.0330	Medium	2.22±0.14	Medium
D	0.682±0.0410	Long	2.90±0.19	Medium
G	0.652±0.0380	Medium	2.92±0.18	Medium
H	0.761±0.0280	Extra long	3.38±0.28	Slender
K	0.567±0.0300	Medium	2.33±0.17	Medium
Black rice				
L	0.595±0.0480	Medium	2.32±0.27	Medium
M	0.560±0.0700	Medium	2.29±0.49	Medium

L/W: Length/width

DISCUSSION

In this study, 13 PR varieties were compared from South Sulawesi, Indonesia (Fig. 1), those have different grain dimension and shape (Table 1). Based on IRRI categorization, the length of PR varieties in this study ranges from extra long (H), long (A, C, D and I) and medium (B, E, F, G, J, K, L and M). For the grain shape, almost all of PR varieties are grouped as medium and only one PR variety that have slender grain shape (H). Grain dimension and shape indicate the quality and appearance of rice which are important considerations in rice preferences. In tropical area, such as in Indonesia, it is reported that the long to medium rice is generally preferred by the consumers (Unnevehr *et al.*, 1992). Therefore, the PR varieties used in this study are mostly preferred by the consumers in Indonesia.

A rice seed consists of several parts, i.e. the outer layer, pericarp and embryo and the endosperm. The endosperm comprises of aleurone layer, it is rich in many essential fatty acids, fiber, vitamins and minerals and endosperm proper containing starch. As the pigment is in the outer of pericarp

Table 2: Proximate analysis results of South Sulawesi pigmented rice

Rice varieties	Water	Ash	Lipid	Protein	Fibre	Carbohydrates
Black waxy rice						
A	12.34	1.45	2.70	7.42	0.78	76.11
C	13.12	1.55	2.63	8.37	0.96	74.35
E	13.14	1.56	2.43	7.55	0.67	75.32
F	11.94	1.56	2.78	7.85	0.79	75.88
I	12.14	1.42	1.90	7.40	0.66	77.14
J	11.70	1.62	2.65	7.53	0.99	76.51
Red rice						
B	11.33	2.01	1.06	11.11	0.75	76.08
D	12.86	1.40	2.54	12.34	0.91	73.41
G	13.32	2.13	2.64	7.24	0.90	71.59
H	13.10	2.13	2.03	7.65	0.98	71.61
K	11.68	1.42	2.03	7.65	0.78	76.11
Black rice						
L	12.21	1.19	2.85	9.49	0.98	76.49
M	12.62	1.72	3.05	14.02	0.92	71.29

Table 3: Color determination results of South Sulawesi pigmented rice

Rice varieties	L^*	a^*	b^*
Black waxy rice			
A	18.69±0.59 ^a	4.46±0.66 ^a	0.47±0.25 ^b
C	19.30±1.74 ^a	4.05±3.08 ^a	2.54±1.00 ^{b,c,d}
E	29.48±1.68 ^{b,c}	3.39±1.11 ^a	0.378±0.21 ^{a,b}
F	19.75±2.17 ^a	4.40±1.07 ^a	-2.86±0.62 ^a
I	26.15±4.07 ^b	3.75±2.20 ^a	1.84±0.60 ^{b,c}
J	38.85±3.71 ^e	4.95±3.52 ^{a,b}	5.52±3.2 ^d
Red rice			
B	50.03±6.33 ^f	5.24±3.41 ^{a,b}	5.49±0.46 ^d
D	49.30±1.74 ^f	15.75±0.84 ^d	13.79±2.63 ^c
G	32.66±0.18 ^c	1.61±0.27 ^a	2.01±0.58 ^{b,c,d}
H	35.58±4.26 ^{d,e}	2.30±0.80 ^a	4.23±1.74 ^{b,c,d}
K	49.89±2.70 ^f	10.81±3.88 ^c	13.47±2.38 ^e
Black rice			
L	36.36±4.38 ^{d,e}	2.88±0.84 ^a	5.48±2.87 ^d
M	26.38±2.67 ^b	9.16±4.89 ^{b,c}	3.66±1.96 ^{b,c,d}

Color parameters: L^* : Brightness, a^* : Redness and b^* : Yellowness



Fig. 1(a-m): Thirteen pigmented rice were collected from 3 different zones of South Sulawesi i.e. 2 areas in Western Zone: Jeneponto regency [(a) Black waxy rice, (b) Non waxy red rice] and Pangkep regency, [(c) Black waxy rice, (d) Non waxy red rice], two areas in Eastern Zone: Sidrap regency, [(e) Black waxy rice] and Sinjai regency, [(f) Black waxy rice, (g) Non waxy red rice, (h) Non waxy red rice] and one area in Transition Zone: TanaToraja regency, [(i) Black waxy rice/black Lalodo, (j) Black waxy rice/black Kobok, (k) Non waxy red rice/red Lea, [(l) Non waxy black rice/black Ambo Tanduk and (m) Non waxy black rice/black Ambo Awok]

layer (Juliano, 1993), rice was collected in the form of paddy and pounded manually prior to analysis. It prevented the loss of pigments during grinding and avoided the damage due to excessive exposure to the sunlight. Once the crop was harvested, the drying process should be conducted immediately to keep moisture level close to 14% (Dipti *et al.*, 2003). The water content in pigmented rice (11.33-13.32%) is expected to maintain the stability of rice during storage. The ash content is the total amount of an inorganic residue from ignition process. It contains a wide variety of minerals derived

from both of the rice tissue itself (physiologic-ash) and the residue of tracer elements (e.g. sand and soil) attached to the rice surface (un-physiologic-ash). Total ash contents in all rice varieties were between 1.42-1.62% (Table 2) but in the present research, the rice mineral contents have not been checked, so it could not claim whether the total ash is physiological ash or un-physiological ash.

Lipid, protein and carbohydrate compositions in all rice varieties were between 1.06-2.78, 7.40-14.02 and 71.29-77.14%, respectively (Table 2). These levels were

almost similar with pigmented rice from Southern Thailand (lipid and protein in the range of 1.44-1.93 and 6.63-8.46%, respectively) (Yodmanee *et al.*, 2011) and Western Indonesia (lipid, protein and carbohydrate between 0.43-0.66, 8.68-10.55 and 87.46-89.28%, respectively) (Indrasari *et al.*, 2010).

The crude fiber contents of samples (0.66-0.99%) (Table 2) were higher than crude fiber contents of pigmented rice grown in Southern Thailand (0.16-0.35%) (Yodmanee *et al.*, 2011) and close to some rice varieties from Western Indonesia (0.57-1.14%) (Indrasari *et al.*, 2010). The higher-fiber content, the better it is as a food supplement. Ambo awok variety was the black rice with the highest nutritional value.

The brightness (L^*), redness (a^*) and yellowness (b^*) values of pigmented rice are indicators for its pigment component. These values were generally low in black rice and black glutinous rice depending on their anthocyanin content and genotype (Yodmanee *et al.*, 2011). The darkest rice had the lowest L^* as shown by black glutinous rice A, C and F varieties, except of J (black kobok) variety which was brighter. The highest and the lowest a^* (redness) values were exhibited by D and G varieties, respectively (Table 3).

Anthocyanins are abundant not only in violet and red fruits but also in colored grains. Abdel-Aal *et al.* (2006) reported that anthocyanin contents in black rice can reach as high as $25000 \mu\text{g g}^{-1}$, bigger than vegetables and fruits ($200\text{-}10000 \mu\text{g g}^{-1}$) depending on its planting condition.

The amount of extracts produced by ethanolic-HCl extraction was varied. The highest and the lowest yields of extracts were A and B, consecutively (Table 4). These extraction yield were positively correlated with both of phenolic content ($r = 0.868$) and anthocyanin content ($r = 0.849$). It also suggested that ethanol solution can be used to extract the polar components of rice, especially anthocyanin. Methanol, ethanol and acetone are suitable extraction solution for anthocyanin and acidic condition needed because anthocyanin is more stable in acid (Giusti and Wroistad, 2001; Rodriguez-Saona and Wroistad, 2001).

Anthocyanin contents were measured using pH differential method. At pH 1.0, anthocyanin is in colored oxonium or flavilium form. While at pH 4.5, anthocyanin is in uncolored carbinol form. Total anthocyanins were calculated by subtracting absorbance at a maximum wavelength of sample solution in two different pH of buffer (Wrolstad, 1993). Black gelatinous rice from western, eastern and transition zones showed higher anthocyanin and phenolic contents than black and red rice. Anthocyanin yields were $94.70\text{-}202.46 \text{ mg Cy-3-glc/100 g db}$ for black glutinous, $66.08\text{-}113.83 \text{ mg Cy-3-glc/100 g db}$ for black rice and $0\text{-}12.85 \text{ mg Cy-3-glc/100 g db}$ for red rice. While phenolic contents of black glutinous, black and red rice were $292.74\text{-}746.25 \text{ mg GAE/100 g db}$, $119.74\text{-}230.10 \text{ mg GAE/100 g db}$ and $12.52\text{-}64.52 \text{ mg GAE/100 g db}$, consecutively (Table 4). These data were similar with the results reported by Sangkitikomol *et al.* (2010a, b) and Abdel-Aal *et al.* (2006).

Anthocyanins exert their antioxidant capacity due to their phenolic groups thereby can be used to treat a wide range of diseases caused by free radicals. Antioxidant activity test showed that black glutinous rice type had the highest DPPH free radical scavenging ability followed by this decreasing order $A > I$ (black Lalodo) = J (black Kobok) $> F$. K (red Lea) harvested from transition zone had a higher antioxidant activity than the other red rice and not significantly different with black glutinous rice C, L (black Ambo Tanduk) and M (black AmbokAwok), although its polyphenols and anthocyanin levels were low (Table 4). These result exhibited that pigmented rice from transition zone (TanaToraja regency) was more valuable than rice from the other zones. TanaToraja is located in the higher altitude and have a colder climate compared to the other regions. This is consistent with research of Saenjum *et al.* (2012) which stated that the rice bran of rice varieties in the mountainous region have higher anti-free radical DPPH activity. It is presumably due to the presence of other antioxidant compounds extracted by acidic ethanol, among other vitamins and unsaturated oils, so it is necessary to analyze other antioxidant components in the extracts.

Table 4: Chemical composition and antioxidant activity of South Sulawesi pigmented rice

Rice varieties	Ethanolic-HCl extraction yield	Polyphenol (mg GAE/100 g db)	Anthocyanin (mg Cy-3-glc/100 g db)	DPPH scavenging activity (%)
Black waxy rice				
A	4.33±0.69	746.25±4.12 ^j	202.46±32.30 ^g	55.70±1.72 ^h
C	2.87±0.62	372.66±15.26 ^f	94.70±1.590 ^c	23.82±1.29 ^{d,e}
E	2.89±0.01	430.11±17.97 ^g	170.71±7.140 ^{e,f}	21.02±2.54 ^{c,d}
F	3.64±0.40	292.74±5.560 ^e	185.36±11.07 ^{f,g}	31.91±2.52 ^f
I	3.38±0.42	564.57±4.050 ^h	140.27±19.41 ^d	49.87±3.57 ^g
J	3.63±0.18	704.33±25.29 ⁱ	188.04±11.06 ^{f,g}	50.31±0.88 ^g
Red rice				
B	1.16±0.08	12.52±4.00 ^a	-	16.54±2.36 ^{a,b}
D	1.59±0.25	64.52±4.19 ^b	10.15±1.12 ^a	19.56±0.55 ^{b,c}
G	1.83±0.08	22.12±0.96 ^a	-	15.34±0.56 ^a
H	1.75±0.14	26.54±1.91 ^a	6.57±0.22 ^a	15.78±0.12 ^a
K	2.32±0.12	51.25±4.60 ^b	12.85±2.29 ^a	26.56±2.65 ^c
Black rice				
L	1.58±0.13	119.74±9.00 ^c	66.08±3.61 ^b	22.22±1.05 ^{c,d}
M	2.79±0.10	230.10±2.30 ^d	113.83±28.68 ^{d,e}	23.06±2.41 ^{c,d}

Activity-based correlation analysis showed that the antioxidant activity was positively correlated with the phenolic ($r = 0.886$) and anthocyanin contents ($r = 0.650$). Similar results were reported by Chakuton *et al.* (2012), Abdel-Aal *et al.* (2006) and Park *et al.* (2008). The antioxidant activity was also significantly correlated with the color of the rice. Rice pigments in the aleurone layer are marked with blackish color because they contain a variety of anthocyanin. The L^* values showed a negative correlation with anthocyanin contents ($r = -0.673$), as well as the total phenolic contents ($r = -0.559$) and antioxidant activity ($r = -0.375$). It means that rice with darkest color and the lowest L^* value can be used as an indicator in selecting rice with high anthocyanin contents and antioxidant activity (Yodmanee *et al.*, 2011).

Based on this study, it can be concluded that pigmented rice varieties from transition zone of South Sulawesi have the best performance and the black glutinous rice has the potential to be developed as a functional food.

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